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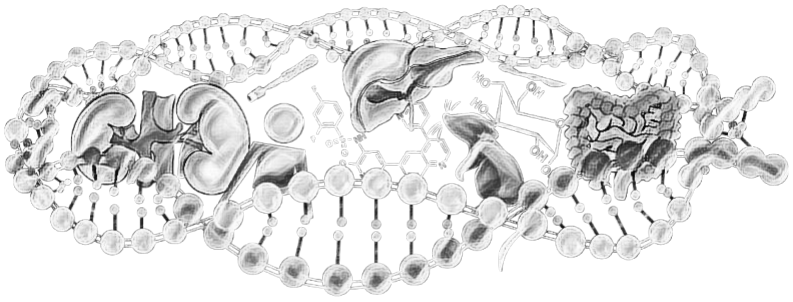
Introduction and aim of this thesis



Introduction

Diseases leading to fibrosis are an increasing clinical and economical burden. It is estimated that fibrosis contributes to almost 45% of the deaths in the developed world [1], as this pathology is a feature of numerous conditions across multiple organs. Fibrosis is an exaggerated wound-healing response to chronic tissue damage. Tissue injury can be caused by several stimuli, such as persistent viral infections, chemicals, autoimmune reactions, radiation and mechanical damage [1]. The physiological response in case of tissue damage is repair, when different processes are activated to restore the architecture and function of the organ. The repair process is driven by various mediators, such as cytokines and chemokines, growth factors and proteolytic enzymes [2,3]. Consequently, chronic damage can lead to an abnormal repair process, and normal parenchymal tissue is replaced by extracellular matrix (ECM). The excess deposition of ECM in an organ represents fibrosis. Although fibrosis is an evolutionarily conserved adaptive process, it is not clear what are the advantages for the organism's survival [4], since with time, all these changes can lead to organ dysfunction, failure and death.

As previously mentioned, the burden of fibrotic diseases is aggravating, as the prevalence of several conditions that are associated with organ fibrosis are increasing. For example, diabetes can lead to kidney fibrosis [5], whereas non-alcoholic fatty liver disease (NAFLD) contributes to both liver and kidney fibrosis [6,7]. NAFLD has become the most common liver disease worldwide due to changes in lifestyle and alimentation [7]. This pathology comprises of a spectrum of disorders, varying from simple steatosis (fatty liver) to a more severe process – non-alcoholic steatohepatitis (NASH), which is characterized by inflammation and hepatocyte damage that can progress to fibrosis, cirrhosis and hepatocellular carcinoma [8].

Fibrosis is recognized as an important cause of morbidity and mortality in many chronic inflammatory diseases, but no treatment that effectively targets this pathogenesis is available on the market. Nevertheless, fibrosis could be reversed if the causal factor is removed, as shown in animal experiments [9] and patients: viral-induced liver fibrosis and cirrhosis receded after inhibition of viral replication was achieved with antiviral agents [10]. However, in the case of patients with very advanced stages of fibrosis, even if the main pathology is treated, fibrosis might not be reversible, as cross-links of fibrillar collagen make the fibrotic scar resistant to enzymatic degradation [11]. Currently, there are numerous studies for the development of new compounds for fibrosis diseases and many molecules with different targets are tested in clinical trials [12]. In the field of liver fibrosis, NASH is currently of high interest, since it is expected to become the most common indication of liver transplantation [13]. This is a result of the recent success in treating and

curing hepatitis C [14], the current leading cause of transplantation in USA and Europe [15]. Encouraging results were obtained with current investigated NASH therapeutics, and several compounds are in Phase II and III clinical trials [16]. Two of the leading contenders for NASH treatment are elafibranor (NCT02704403) and obeticholic acid (NCT02548351), both in Phase III clinical trials. These drugs are targeting the reduction of hepatic fat accumulation. Elafibranor is an agonist of peroxisome proliferator-activator receptors (PPAR) α/δ, two nuclear receptors that modulate lipid metabolism by enhancing fatty acid transport and oxidation; in addition, their activation has anti-inflammatory effects and increase the insulin sensitivity [17]. Obeticholic acid is a ligand for farnesoid X receptor (FXR), a nuclear receptor that negatively regulates the synthesis of bile acid, decreases hepatic lipogenesis and steatosis [18], and improves peripheral insulin sensitivity [19].

Numerous immunological and molecular mechanisms can contribute to development and progression of fibrosis [20]. The main contributors to fibrosis are the innate and adaptive immune responses, together with exaggerated ECM production by myofibroblasts. These aspects should be taken into consideration for the design of new treatments. Although currently there are clinical developments in fibrosis research, future treatments options might involve combination regimens that address the primary disease and also inflammation and/or fibrosis mediators and pathways.

Fibrosis models

To address the need for drug development of antifibrotic compounds, predictive preclinical models that can improve clinical translation are essential. Although *in vivo* animal experiments remain the most used preclinical models, relevant animal models are still lacking in the field of fibrosis research [21–24]. In spite of an increase in number, variety and sophistication of animal models of fibrosis [21,23,24], the overall comparability to the human disease is weak. *In vitro* models represent another type of preclinical research. Several *in vitro* models were developed to ensure faster analysis and high throughput screening, but their relevance is also limited [25,26]. The most common *in vitro* models used for the study of fibrosis are: cell culture of rodent and human cell lines, culture of primary human fibroblasts, precision-cut tissue slices, co-culture systems, microfluidic-based bioreactor cultures, 3D bioprinted tissue and organoids [27–30]. The *in vitro* models have the advantage of using also human cells/tissues, eliminating the need for interspecies translation and higher relevance for the human disease.

PCTS

In the past years, several studies showed the relevance of precision-cut tissue slices (PCTS) in the study of organ fibrosis [31–34]. PCTS represent a 3D model

of an organ, where the original architecture and cellular composition is preserved, maintaining all cell-cell and cell-matrix interactions. PCTS are prepared from fresh organs obtained from sacrificed animals or human surgical waste tissue. The advantages of using this model include the use of several organs from one animal, the possibility of testing more drug concentrations in the same organ and using the same animal as its own control. Furthermore, the use of PCTS allows the study of an organ-specific reaction to a pharmaceutical compound, eliminating the effect of the infiltrating immune cells. PCTS are the best representation of an organ *ex vivo*, since the organ's morphology and structure are not disrupted. The interest for PCTS in the study of fibrosis is due to the observation that PCTS preparation (two-cut surfaces) and culture triggers a spontaneous fibrotic response, characterized by production of ECM components and myofibroblast activation (as shown by the increased protein expression of α smooth muscle actin) [34]. Several studies showed that PCTS can be used to assess the antifibrotic effect of small molecules, targeting different receptors and pathways [31,33,35–39], such as tyrosine kinases. Additionally, human liver PCTS were successfully used to investigate the downstream effect of the anti-NASH compound, obeticholic acid [40]. However, PCTS are still a model and therefore it is not the exact replication of a disease and not all characteristics of that condition will be reproduced.

The comparison between *in vivo* results from animals or patients and *ex vivo* murine or human PCTS results can determine the value of PCTS in fibrosis research. The optimal drug to test would be an efficient antifibrotic drug with known mechanism of action. Unfortunately, the absence of an efficient proven antifibrotic drug limits the comparison. Nevertheless, several drugs with different targets are in preclinical and clinical studies [12]. These drugs can be assessed in rodent and human PCTS and compared to *in vivo* results, in order to evaluate the correlation between ex vivo and in vivo data. The use of human tissue would eliminate the murine – human translation, while making the results more relevant for the human disease and possibly preventing toxicity and side effects in clinical trials. PCTS could help to identify ineffective treatments in order to concentrate valuable resources on other potential drug candidates.

Although the use of PCTS slices has increased, the processes during culture are not completely elucidated. A better understanding of the biological processes that occur during culture will allow us to acknowledge for which diseases PCTS may have predictive capacity, which molecules and signaling pathways are the main drivers of the condition developed by PCTS, and how we can optimize culture conditions to prolong the viability of slices. Therefore, it is essential to perform a comprehensive study to assess which fibrosis mechanisms are involved PCTS due to preparation and culture. PCTS viability depends on the organ and species of origin,

and most studies use 48h culture, since the viability of mouse and human slices is maintained during this time frame. Considering the limited incubation time, most of the changes induced by culture can be observed on transcriptional level. The question regarding the on-going complex processes during PCTS culture could be answered using an advanced sequencing technology: next-generation sequencing (NGS). This technique was developed more than a decade ago and allows the profiling of the whole transcriptome by determining gene and transcript abundance. Currently, there are available several companies and technologies that can perform next-generation sequencing, but we used Illumina. The Illumina technology uses sequencing by synthesis chemistry and can use DNA or complementary DNA [41,42]. Translating the abundance of data resulted from NGS into biological context can be challenging. The interpretation of this data can be done with Ingenuity® Pathway Analysis (IPA), a powerful tool designed to predict downstream effects on biological processes and identify key regulators of these processes. Additionally, the NGS data can reveal new targets or potential biomarkers for particular conditions.

Biomarker/testing

Drug development of antifibrotic compounds is a challenging process, as there is no biomarker/test that allows a safe and accurate measurement of small changes in fibrosis during clinical trials. The ideal biomarker meets the following requirements: organ specific, sensitive to disease regression or progression, easy accessible with a minimal invasive procedure and cost effective [43]. Unfortunately, the ideal biomarker for fibrotic diseases is not discovered yet. Nevertheless, there are several methods used to assess the presence of fibrosis and the approximate amount of fibrosis. These methods include liver biopsy and morphological assessment of pathology specimens, and FibroTest for liver [44], magnetic resonance imaging and ECM components analysis for several organs, such as liver, intestine and kidney [44–46]. ECM is an insoluble scaffold and it is composed of fibrillar and non-fibrillar collagens, elastic fibers and glycoproteins [47]. The most abundant ECM protein, collagen, is the result of a complex process that includes biosynthesis, assembly and crosslinking [48]. Several signaling pathways can mediate this process and its regulation can occur post-transcriptional or post-translational [48]. To assess if fibrosis is advancing, it is important to evaluate the formation of new ECM. Collagen is synthesized as a precursor molecule that has two propeptide extensions at the N-terminal and C-terminal [49]. The propeptides are cleaved by proteinases, releasing the fibril-forming, mature form of collagen [49]. Therefore, measuring the serum levels of one of the propeptides – procollagen type I N-terminal propeptide (PINP), was proposed as a marker of fibrogenesis [50]. However, there is also a limitation for using this marker, since PINP can be cleared by liver endothelial cells and can be increased

when these cells are damaged [50]. The complex process of collagen production is difficult to evaluate and the changes observed on gene expression or soluble protein-levels might not reflect the changes in ECM architecture.

Considering that fibrosis biomarkers are necessary not only as an indicator for treatment response in drug development, but also for identifying which patients are at risk for disease progression and organ failure, it is imperative to identify and validate proper biomarkers.

Aim and Scope of the thesis

The research described in this thesis is aimed at exploring the mechanism of fibrosis progression and reversal in different organs using PCTS. Fibrosis has several stages, namely initiation, progression, end-stage and in the case of successful treatment, a resolution phase. PCTS mimic the initiation and progression of fibrosis due to their preparation and culture [37,51,52]. The possibility of using both healthy and diseased tissues to prepare PCTS allowed us to investigate the early and late stages of fibrosis. We also investigated different etiologies in inducing liver fibrosis murine models, including diet-induced NASH. The scope of the thesis was to expand the understanding of the biological process that characterize the culture period, together with development of an *ex vivo* NASH murine model and drug testing of antifibrotic and anti-NAFLD compounds. The optimization of PCTS will also address the ethical concerns regarding laboratory animals based on the 3Rs principles - "Reduction, Refinement and Replacement".

The chapters in this thesis are aimed at increasing our understanding of PCTS and assessing their potential for fibrosis research. The type of study and tissue used are presented in Figure 1. In **Chapter 2** we use NGS and IPA to describe the transcriptional changes and biological processes that occur during culture of healthy murine and human PCTS from different organs (liver, kidney, jejunum, ileum and colon), with focus on inflammation and fibrosis. To elucidate species- and organ-differences, we compared the two species with regard to their response to culture. **Chapter 3** continues the investigation into transcriptional changes during culture for healthy and diseased human PCTS obtained from three organs: liver, kidney and ileum. Additionally, we investigated the levels of different cytokines released in culture media to compare the two models: healthy PCTS (model for early fibrosis) and diseased PCTS (model for late-stage fibrosis). We expect that the knowledge from **Chapter 2** and **3** will allow us to improve culture conditions, extend PCTS viability and identify possible targets and biomarkers for the treatment of fibrosis. Next, in **Chapter 4**, we use NAFLD animal models to obtain steatotic liver PCTS and evaluate their potential as an *ex vivo* NASH model. We also tested whether inflammation and fibrosis can be further induced in this model by using specific modulators. The last part of this study included drug testing of a possible anti-NAFLD compound, elafibranor and the comparison of its effects in slices and *in vivo* in mice. The following study, **Chapter 5**, investigates the potential antifibrotic effect resulted from the inhibition of the PI3K signaling pathway. This pathway can be activated by a plethora of mediators involved in fibrosis development; therefore, it is emerging as a promising therapeutic target. For this purpose we evaluated the effects of a PI3K inhibitor, omipalisib in healthy and diseased murine and human

liver slices. In addition, we evaluated a potential biomarker for NASH: Glycoprotein Nonmetastatic Melanoma Protein B (GPNMB) in **Chapter 6**, as progress in the biomarker field is crucial for the advance of fibrosis research. For this we assessed the transcriptional profile of GPNMB in liver slices from murine NASH and fibrosis models and human healthy and cirrhotic livers. Finally, **Chapter 7** provides a general summary of the obtained results and discusses the implication of these results, together with future perspectives of using PCTS.

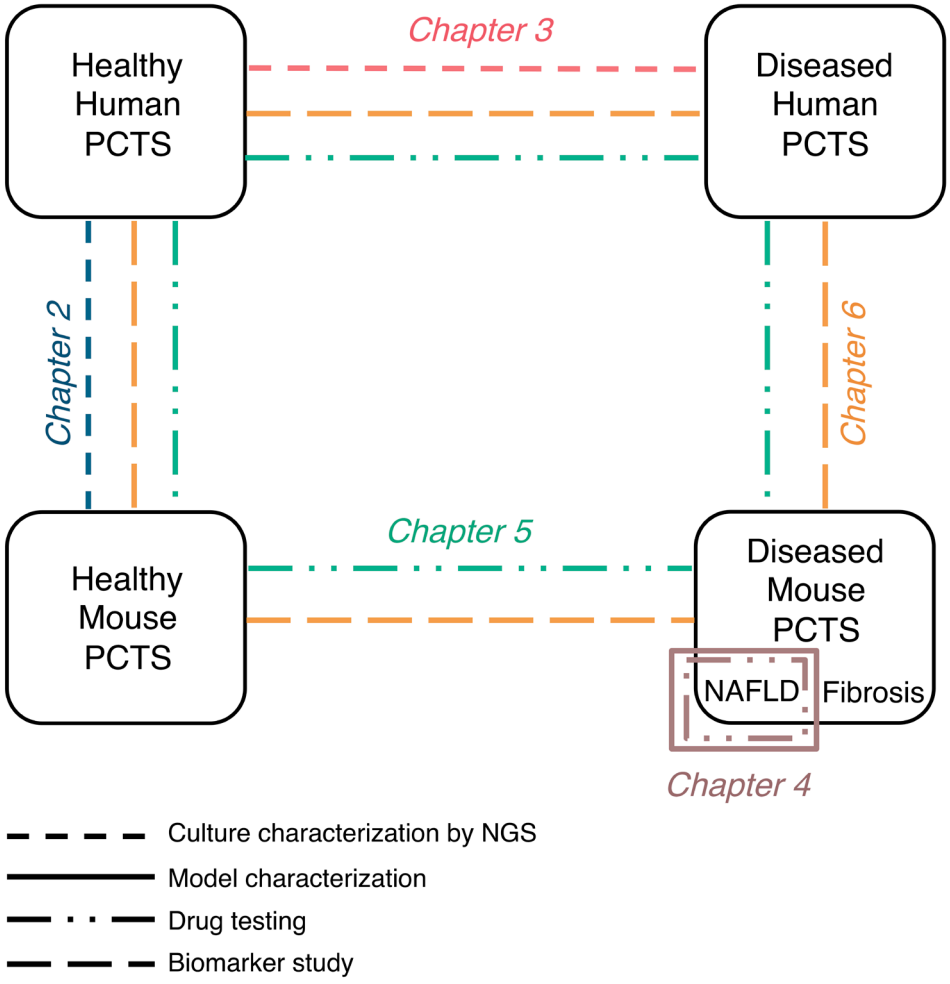


Figure 1. Studies and tissue types used across the thesis. PCTS – precision-cut tissue slices, NAFLD – non-alcoholic fatty liver disease, NGS – next-generation sequencing.

References

[1] T. Wynn, Cellular and molecular mechanisms of fibrosis, J. Pathol. 214 (2008) 199–210. doi:10.1002/path.2277.

[2] T. a Wynn, Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases, J. Clin. Invest. 117 (2007) 524–529. doi:10.1172/JCI31487.

[3] J.J. Tomasek, G. Gabbiani, B. Hinz, C. Chaponnier, R.A. Brown, Myofibroblasts and mechano-regulation of connective tissue remodelling., Nat. Rev. Mol. Cell Biol. 3 (2002) 349–63. doi:10.1038/nrm809.

[4] V.J. Thannickal, Y. Zhou, A. Gaggar, S.R. Duncan, Fibrosis: ultimate and proximate causes, J. Clin. Invest. 124 (2014) 4673–4677. doi:10.1172/JCI74368.

[5] M. Abbate, C. Zoja, G. Remuzzi, How Does Proteinuria Cause Progressive Renal Damage?, J. Am. Soc. Nephrol. 17 (2006) 2974–2984. doi:10.1681/ASN.2006040377.

[6] H.R. Jang, D. Kang, D.H. Sinn, S. Gu, S.J. Cho, J.E. Lee, W. Huh, S.W. Paik, S. Ryu, Y. Chang, T. Shafi, M. Lazo, E. Guallar, J. Cho, G.-Y. Gwak, Nonalcoholic fatty liver disease accelerates kidney function decline in patients with chronic kidney disease: a cohort study, Sci. Rep. 8 (2018) 4718. doi:10.1038/s41598-018-23014-0.

[7] Z. Younossi, Q.M. Anstee, M. Marietti, T. Hardy, L. Henry, M. Eslam, J. George, E. Bugianesi, Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention, Nat. Rev. Gastroenterol. Hepatol. 15 (2017) 11–20. doi:10.1038/nrgastro.2017.109.

[8] S.L. Friedman, B.A. Neuschwander-Tetri, M. Rinella, A.J. Sanyal, Mechanisms of NAFLD development and therapeutic strategies, Nat. Med. 24 (2018) 908–922. doi:10.1038/s41591-018-0104-9.

[9] R. Issa, Apoptosis of hepatic stellate cells: involvement in resolution of biliary fibrosis and regulation by soluble growth factors, Gut. 48 (2001) 548–557. doi:10.1136/gut.48.4.548.

[10] P. Marcellin, E. Gane, M. Buti, N. Afdhal, W. Sievert, I.M. Jacobson, M.K. Washington, G. Germanidis, J.F. Flaherty, R.A. Schall, J.D. Bornstein, K.M. Kitrinos, G.M. Subramanian, J.G. McHutchison, E.J. Heathcote, Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study, Lancet. 381 (2013) 468–475. doi:10.1016/S0140-6736(12)61425-1.

[11] V. Hernández-Gea, Liver Fibrosis: What Is Reversible and What Not? How to Assess Regression?, in: Portal Hypertens. VI, Springer International Publishing, Cham, 2016: pp. 111–115. doi:10.1007/978-3-319-23018-4_14.

[12] C.B. Nanthakumar, R.J.D. Hatley, S. Lemma, J. Gauldie, R.P. Marshall, S.J.F. Macdonald, Dissecting fibrosis: therapeutic insights from the small-molecule toolbox, Nat. Rev. Drug Discov. 14 (2015) 693–720. doi:10.1038/nrd4592.

[13] K. Stephenson, L. Kennedy, L. Hargrove, J. Demieville, J. Thomson, G. Alpini, H. Francis, Updates on Dietary Models of Nonalcoholic Fatty Liver Disease: Current Studies and Insights, Gene Expr. 18 (2018) 5–17. doi:10.3727/105221617X15093707969658.

[14] J.L. Horsley-Silva, H.E. Vargas, New Therapies for Hepatitis C Virus Infection., Gastroenterol. Hepatol. (N. Y). 13 (2017) 22–31. http://dx.doi.org/10.1016/B978-0-12-387040-7.00003-2.

[15] S. Martini, Hepatitis C and liver transplantation., Minerva Gastroenterol. Dietol. 64 (2018) 158–169. doi:10.23736/S1121-421X.17.02448-5.

[16] J.J. Connolly, K. Ooka, J.K. Lim, Future Pharmacotherapy for Non-alcoholic Steatohepatitis (NASH): Review of Phase 2 and 3 Trials, J. Clin. Transl. Hepatol. 6 (2018) 1–12. doi:10.14218/JCTH.2017.00056.

[17] V. Ratziu, S.A. Harrison, S. Francque, P. Bedossa, P. Lehert, L. Serfaty, M. Romero-Gomez, J. Boursier, M. Abdelmalek, S. Caldwell, J. Drenth, Q.M. Anstee, *et al*, Elafibranor, an Agonist of the Peroxisome Proliferator–Activated Receptor–α and –δ, Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening, Gastroenterology. 150 (2016) 1147-1159.e5. doi:10.1053/j.gastro.2016.01.038.

[18] M. Watanabe, S.M. Houten, L. Wang, A. Moschetta, D.J. Mangelsdorf, R.A. Heyman, D.D. Moore, J. Auwerx, Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c, J. Clin. Invest. 113 (2004) 1408–1418. doi:10.1172/JCI21025.

[19] G. Porez, J. Prawitt, B. Gross, B. Staels, Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease, J. Lipid Res. 53 (2012) 1723–1737. doi:10.1194/jlr.R024794.

[20] T.A. Wynn, T.R. Ramalingam, Mechanisms of fibrosis: therapeutic translation for fibrotic disease, Nat. Med. 18 (2012) 1028–1040. doi:10.1038/nm.2807.

[21] S. Crespo Yanguas, B. Cogliati, J. Willebrords, M. Maes, I. Colle, B. van den Bossche, C.P.M.S. de Oliveira, W. Andraus, V.A. Alves, I. Leclercq, M. Vinken, Experimental models of liver fibrosis, Arch. Toxicol. 90 (2016) 1025–1048. doi:10.1007/s00204-015-1543-4.
[22] J. Tashiro, G.A. Rubio, A.H. Limper, K. Williams, S.J. Elliot, I. Ninou, V. Aidinis, A. Tzouvelekis, M.K. Glassberg, Exploring Animal Models That Resemble Idiopathic Pulmonary Fibrosis, Front. Med. 4 (2017) 1–11. doi:10.3389/fmed.2017.00118.
[23] F. Rieder, S. Kessler, M. Sans, C. Fiocchi, Animal models of intestinal fibrosis: new tools for the understanding of pathogenesis and therapy of human disease, Am. J. Physiol. Liver Physiol. 303 (2012) G786–G801. doi:10.1152/ajpgi.00059.2012.
[24] A. Nogueira, M.J. Pires, P.A. Oliveira, Pathophysiological Mechanisms of Renal Fibrosis: A Review of Animal Models and Therapeutic Strategies, In Vivo (Brooklyn). 31 (2017) 1–22. doi:10.21873/invivo.11019.
[25] G.P. Smith, Animal Models of Fibrosis in Human Disease, in: Anim. Model. Study Hum. Dis., Elsevier, 2013: pp. 435–458. doi:10.1016/B978-0-12-415894-8.00019-1.
[26] M. Asmani, S. Velumani, Y. Li, N. Wawrzyniak, I. Hsia, Z. Chen, B. Hinz, R. Zhao, Fibrotic microtissue array to predict anti-fibrosis drug efficacy, Nat. Commun. 9 (2018) 2066. doi:10.1038/s41467-018-04336-z.
[27] A. Sundarakrishnan, Y. Chen, L.D. Black, B.B. Aldridge, D.L. Kaplan, Engineered cell and tissue models of pulmonary fibrosis, Adv. Drug Deliv. Rev. 129 (2018) 78–94. doi:10.1016/j.addr.2017.12.013.
[28] L.A. van Grunsven, 3D in vitro models of liver fibrosis, Adv. Drug Deliv. Rev. 121 (2017) 133–146. doi:10.1016/j.addr.2017.07.004.
[29] E.S. Rodansky, L.A. Johnson, S. Huang, J.R. Spence, P.D.R. Higgins, Intestinal organoids: A model of intestinal fibrosis for evaluating anti-fibrotic drugs, Exp. Mol. Pathol. 98 (2015) 346–351. doi:10.1016/j.yexmp.2015.03.033.
[30] T.M. DesRochers, E. Palma, D.L. Kaplan, Tissue-engineered kidney disease models, Adv. Drug Deliv. Rev. 69–70 (2014) 67–80. doi:10.1016/j.addr.2013.12.002.
[31] E.G.D. Stribos, T. Luangmonkong, A.M. Leliveld, I.J. de Jong, W.J. van Son, J.-L. Hillebrands, M.A. Seelen, H. van Goor, P. Olinga, H.A.M. Mutsaers, Precision-cut human kidney slices as a model to elucidate the process of renal fibrosis, Transl. Res. 170 (2016) 8-16.e1. doi:10.1016/j.trsl.2015.11.007.
[32] E.G.D. Stribos, M.A. Seelen, H. van Goor, P. Olinga, H.A.M. Mutsaers, Murine Precision-Cut Kidney Slices as an ex vivo Model to Evaluate the Role of Transforming Growth Factor-β1 Signaling in the Onset of Renal Fibrosis, Front. Physiol. 8 (2017) 1–9. doi:10.3389/fphys.2017.01026.
[33] H.N. Alsafadi, C.A. Staab-Weijnitz, M. Lehmann, M. Lindner, B. Peschel, M. Königshoff, D.E. Wagner, An ex vivo model to induce early fibrosis-like changes in human precision-cut lung slices, Am. J. Physiol. Cell. Mol. Physiol. 312 (2017) L896–L902. doi:10.1152/ajplung.00084.2017.
[34] T. Luangmonkong, S. Suriguga, E. Bigaeva, M. Boersema, D. Oosterhuis, K.P. de Jong, D. Schuppan, H.A.M. Mutsaers, P. Olinga, Evaluating the antifibrotic potency of galunisertib in a human ex vivo model of liver fibrosis, Br. J. Pharmacol. 174 (2017) 3107–3117. doi:10.1111/bph.13945.
[35] I.M. Westra, H.A.M. Mutsaers, T. Luangmonkong, M. Hadi, D. Oosterhuis, K.P. de Jong, G.M.M. Groothuis, P. Olinga, Human precision-cut liver slices as a model to test antifibrotic drugs in the early onset of liver fibrosis, Toxicol. Vitr. 35 (2016) 77–85. doi:10.1016/j.tiv.2016.05.012.
[36] T. Luangmonkong, S. Suriguga, A. Adhyatmika, A. Adlia, D. Oosterhuis, C. Suthisisang, K.P. de Jong, H.A.M. Mutsaers, P. Olinga, In vitro and ex vivo anti-fibrotic effects of LY2109761, a small molecule inhibitor against TGF-β, Toxicol. Appl. Pharmacol. 355 (2018) 127–137. doi:10.1016/j.taap.2018.07.001.
[37] R. Iswandana, B.T. Pham, W.T. van Haaften, T. Luangmonkong, D. Oosterhuis, H.A.M. Mutsaers, P. Olinga, Organ- and species-specific biological activity of rosmarinic acid, Toxicol. Vitr. 32 (2016) 261–268. doi:10.1016/j.tiv.2016.01.009.
[38] M. Cedilak, M. Banjanac, D. Belamarić, A. Paravić Radičević, I. Faraho, K. Ilić, S. Čužić, I. Glojnarić, V. Eraković Haber, M. Bosnar, Precision-cut lung slices from bleomycin treated animals as a model for testing potential therapies for idiopathic pulmonary fibrosis, Pulm. Pharmacol. Ther. 55 (2019) 75–83. doi:10.1016/j.pupt.2019.02.005.
[39] M. Lehmann, L. Buhl, H.N. Alsafadi, S. Klee, S. Hermann, K. Mutze, C. Ota, M. Lindner, J. Behr, A. Hilgendorff, D.E. Wagner, M. Königshoff, Differential effects of Nintedanib and Pirfenidone on lung alveolar epithelial cell function in ex vivo murine and human lung tissue

cultures of pulmonary fibrosis, Respir. Res. 19 (2018) 175. doi:10.1186/s12931-018-0876-y.
[40] N. Ijssennagger, A.W.F. Janssen, A. Milona, J.M. Ramos Pittol, D.A.A. Hollman, M. Mokry, B. Betzel, F.J. Berends, I.M. Janssen, S.W.C. Van Mil, S. Kersten, Gene expression profiling in human precision cut liver slices in response to the FXR agonist obeticholic acid, J. Hepatol. 64 (2016) 1158–1166. doi:10.1016/j.jhep.2016.01.016.
[41] M.G. Ross, C. Russ, M. Costello, A. Hollinger, N.J. Lennon, R. Hegarty, C. Nusbaum, D.B. Jaffe, Characterizing and measuring bias in sequence data, Genome Biol. 14 (2013) R51. doi:10.1186/gb-2013-14-5-r51.
[42] D.R. Bentley, S. Balasubramanian, H.P. Swerdlow, G.P. Smith, J. Milton, C.G. Brown, K.P. Hall, D.J. Evers, C.L. Barnes, H.R. Bignell, J.M. Boutell, J. Bryant, *et al*, Accurate whole human genome sequencing using reversible terminator chemistry, Nature. 456 (2008) 53–59. doi:10.1038/nature07517.
[43] M. Pinzani, K. Rombouts, S. Colagrande, Fibrosis in chronic liver diseases: diagnosis and management, J. Hepatol. 42 (2005) S22–S36. doi:10.1016/j.jhep.2004.12.008.
[44] K.S. Nallagangula, S.K. Nagaraj, L. Venkataswamy, M. Chandrappa, Liver fibrosis: a compilation on the biomarkers status and their significance during disease progression, Futur. Sci. OA. 4 (2018) FSO250. doi:10.4155/fsoa-2017-0083.
[45] P. Giuffrida, M. Pinzani, G.R. Corazza, A. Di Sabatino, Biomarkers of intestinal fibrosis – one step towards clinical trials for stricturing inflammatory bowel disease, United Eur. Gastroenterol. J. 4 (2016) 523–530. doi:10.1177/2050640616640160.
[46] S.G. Mansour, J. Puthumana, S.G. Coca, M. Gentry, C.R. Parikh, Biomarkers for the detection of renal fibrosis and prediction of renal outcomes: a systematic review, BMC Nephrol. 18 (2017) 72. doi:10.1186/s12882-017-0490-0.
[47] T.R. Cox, J.T. Erler, Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer, Dis. Model. Mech. 4 (2011) 165–178. doi:10.1242/dmm.004077.
[48] P. Sivakumar, C. Kitson, G. Jarai, Modeling and measuring extracellular matrix alterations in fibrosis: challenges and perspectives for antifibrotic drug discovery, Connect. Tissue Res. 60 (2019) 62–70. doi:10.1080/03008207.2018.1500557.
[49] K. Gelse, Collagens—structure, function, and biosynthesis, Adv. Drug Deliv. Rev. 55 (2003) 1531–1546. doi:10.1016/j.addr.2003.08.002.
[50] S.S. Veidal, E. Vassiliadis, A.-C. Bay-Jensen, G. Tougas, B. Vainer, M.A. Karsdal, Procollagen type I N-terminal propeptide (PINP) is a marker for fibrogenesis in bile duct ligation-induced fibrosis in rats, Fibrogenesis Tissue Repair. 3 (2010) 5. doi:10.1186/1755-1536-3-5.
[51] E.G.D. Stribos, T. Luangmonkong, A.M. Leliveld, I.J. de Jong, W.J. van Son, J.-L. Hillebrands, M.A. Seelen, H. van Goor, P. Olinga, H.A.M. Mutsaers, Precision-cut human kidney slices as a model to elucidate the process of renal fibrosis, Transl. Res. 170 (2016) 8-16.e1. doi:10.1016/j.trsl.2015.11.007.
[52] I.M. Westra, H.A.M. Mutsaers, T. Luangmonkong, M. Hadi, D. Oosterhuis, K.P. de Jong, G.M.M. Groothuis, P. Olinga, Human precision-cut liver slices as a model to test antifibrotic drugs in the early onset of liver fibrosis, Toxicol. Vitr. 35 (2016) 77–85. doi:10.1016/j.tiv.2016.05.012.